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REGULAR ARTICLE

Nitrogen enrichment lowers *Betula pendula* green and yellow leaf stoichiometry irrespective of effects of elevated carbon dioxide

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Abstract Elevated carbon dioxide (CO_2) and increased nitrogen (N) availability generally increase deciduous tree biomass and alter green and yellow leaf stoichiometry. This paper investigates whether this also applies to *Betula pendula* (Birch). The effects of elevated atmospheric CO_2 (600 ppmv) and increased N availability (50 and 100 kg N ha^{-1} $year^{-1}$) of differential inorganic nitrogen type (ammonium, nitrate and ammonium nitrate) on net primary production (NPP) and green/yellow leaf C:N of *Betula pendula* saplings were studied for 3 years. The combination of both factors raised NPP, but elevated CO_2 alone did not. In green leaves, increased N availability raised N concentrations, outweighing decreases caused by elevated CO_2. After senescence, increased N concentrations were found at 100 kg N ha^{-1} $year^{-1}$, also leading to a lower C:N ratio. Although a lower C:N ratio may increase the decomposition rate during early decomposition, it may have the opposite effect during later decomposition stages. This, in combination with increased biomass production and possibly lower soil pH values, might increase soil C storage. However, due to the complexity of soil C formation and related processes this remains unsure.

Keywords Elevated CO_2 · Nitrogen enrichment · N concentration · C:N · Resorption · *Betula pendula*

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Introduction

Since the Industrial Revolution, the concentration of atmospheric carbon dioxide (CO_2) has increased exponentially from approximately 285 ppmv in 1850 to 369 ppmv in 2000 (Friedli et al. 1986; Keeling and Whorf 2005), and for 2100 values between 400 and 1000 ppmv are predicted (Enting et al. 1994; IPCC 2001). Nitrogen oxide (NO_x) and ammonia (NH_y) emissions have also increased since the second half of the nineteenth century and have led to an increase in wet deposition of nitrate (NO_3^-) and ammonium (NH_4^+), dry deposition of gaseous nitric acid, ammonia and NO_x, and particulate NO_3^- (Pitcairn et al. 1995; Freyer et al. 1996; Asman et al. 1998; Erisman et al. 1998b; Holland et al. 1999; Lawrence et al.

2000; Fowler et al. 2004). Despite emission reduction in some parts of Western Europe since the 1990s, nitrogen (N) emission levels are still expected to rise in other parts of the world (Dentener et al. 2006).

The general effects of elevated CO_2 on C_3 plants include increased photosynthesis, water use efficiency, net primary production (NPP), and decreased N concentration (or an increased C:N ratio; Pettersson and McDonald 1992; Ceulemans and Mousseau 1994; Mcguire et al. 1995; Penuelas and Estiarte 1997; Poorter et al. 1997; Curtis and Wang 1998; Gifford et al. 2000; DeLucia and Thomas 2000; Norby et al. 2001; Finzi et al. 2002; Coley et al. 2002). Because N availability limits plant growth in many terrestrial ecosystems, an increase in N availability generally has a positive effect on plant growth and/or biomass, total plant N, area-based plant N concentration, and specific leaf area (Bobbink et al. 1998; Bauer et al. 2001; Lipson and Nasholm 2001; Pal et al. 2005; van den Berg et al. 2005b; Reich et al. 2006).

While the single factor effects of elevated CO_2 and N availability on the physiology of plants are well understood, the effects of combined increases of both resources have not been investigated as thoroughly. Several studies have been undertaken on agricultural crops (Nakamura et al. 1999; Li et al. 2004; Pal et al. 2005 a.o.) or grassland plants and shrubs (van der Heijden et al. 2000; Heijmans et al. 2001; Reich et al. 2006). Less studies have focused on deciduous trees as either seedlings or adults and only a few include birch species (Mcguire et al. 1995; Lippert et al. 1996; Curtis and Wang 1998). In general, both elevated CO_2 and increased N availability were generally found to increase biomass.

Apart from the physiological effects, elevated CO_2 and increased N availability may also influence deciduous leaf and litter stoichiometry. The effects of elevated CO_2 and increased N availability on tree litter quality can be opposite and are therefore not yet fully understood. While yellow leaf C:N may be unaffected or positively affected by elevated CO_2, it may be unaffected to negatively affected in response to increased N availability (Cotrufo et al. 1995; Hirschel et al. 1997; Scherzer et al. 1998; Norby et al. 1999; Gifford et al. 2000; Norby et al. 2001). Some studies on deciduous tree species have investigated both factors in combination, but most often the emphasis was solely on primary production and yellow leaf C:N values have not been reported (Pettersson et al. 1993; Mcguire et al. 1995; Bauer et al. 2001).

Although higher N availability generally stimulates plant growth and biomass, there may be differences in plant responses depending on the chemical composition of the N source. Generally, plants from circumneutral, alkaline, and aerobic soils prefer NO_3^- as their N source, while those from acid and anaerobic soils often prefer NH_4^+ (Gigon and Rorison 1972; de Graaf et al. 1998; van den Berg et al. 2005a). Other authors indicate that plant species of acidic soils where NH_4^+ is usually the predominant form of inorganic N (Troelstra et al. 1990) may be less negatively affected by increased NH_4^+ concentrations, but that they do not perform better on either NH_4^+ or NO_3^- as their sole N source (Gigon and Rorison 1972; Falkengren-Grerup 1995).This is probably caused by differential effects of both N species on plant functioning, e.g., through soil pH or microbial competition and preference (Kaye and Hart 1997; Britto and Kronzucker 2002; Paulissen et al. 2004; van den Berg et al. 2005a; Guo et al. 2007). However, studies on the effects of N enrichment on deciduous trees generally have applied N as NH_4NO_3, combining both NH_4^+ and NO_3^- (Bauer et al. 2001). Only very few studies have made a distinction between NH_4-N and NO_3-N (Crabtree and Bazzaz 1993; Templer and Dawson 2004) and therefore information on the possible differential effects of NH_4^+ and NO_3^- addition on deciduous tree species is very limited.

Both the increase in biomass and changes in green and yellow leaf stoichiometry or N concentration may influence the decomposition process and, finally, soil C storage. Early studies indicate that a higher N concentration in litter would increase the decomposition rate in general (Swift et al. 1979). However, later studies indicate that decomposition is divided into two stages and that macronutrients, such as N, only influence decomposition during the early stage which may last for a few months (Berg 2000). In the late stage, as the concentration of recalcitrant lignin fractions increases, decomposition rates are increasingly suppressed. Here, Berg (2000) found a clearly significant and negative relationship between the initial N concentration of the litter and decomposition. This author indicates that this is probably due to the repressed formation of lingo-lytic enzymes in white-rot fungi by low-molecular N compounds and/or the formation of recalcitrant complexes by the reaction of

ammonia or amino acids with lignin degradation products. In the late stage of decomposition leading towards the formation of soil organic matter, the inhibition of the decomposition rate by increased litter N concentrations may be so strong that decomposition reaches a limit value at which total mass losses virtually stop and contribute to soil C storage (Berg and Meentemeyer 2002).

Reviewing the effects of elevated CO_2 and increased N availability in general is difficult enough across species, but upgrading results towards forest or ecosystem level is even more problematic. At first, most experiments were short-term, and were performed on a small scale with (young) seedlings. Norby et al. (1999) found that most of the results of these experiments are qualitatively correct compared to results of experiments over several growing seasons under field conditions. Furthermore, Körner et al. (2005) indicate that large, mature forest trees respond physiologically to elevated atmospheric CO_2 in a manner similar to the younger trees used in most studies. Many deciduous Northern-Hemisphere forests include or are even dominated by birch species. Because of the relatively small number of studies on this species, the validation of general effects of elevated CO_2 and N availability combined on plant production, leaf physiology and N source performance is very limited, if not absent. The very few studies on Birch specifically may possibly restrain conclusions on the C and N cycles in these systems. Therefore, the present study has focused on birch, *Betula pendula* specifically.

This study addresses the following main research questions. (a) What is the combined effect of increased CO_2 and N enrichment on NPP and yellow leaf stoichiometry of *B. pendula*; and (b) is the response of *B. pendula* to increased N dependent on the proportion of inorganic N species? The following hypotheses have been formulated:

1. Both elevated CO_2 and increased N availability will lead to a higher NPP. Combined, they will lead to an even higher NPP.
2. Yellow leaves grown at elevated CO_2 will have a higher C:N ratio than those grown at ambient CO_2. N enrichment will lead to a lower yellow leaf C:N ratio. Combined, the C:N ratio will be lowered compared to a control situation due to the stronger effect of N enrichment.
3. Because *B. pendula* is a species that characteristically grows on acid and medium-nutritious soils, it will produce more biomass when given NH_4^+ instead of NO_3^-.

To test these hypotheses, a greenhouse experiment was performed with *B. pendula* saplings for a period of 3 years in ambient and elevated CO_2, and with three different N treatments: NH_4^+, NO_3^-, and NH_4NO_3.

Materials and methods

Experimental conditions

The study was carried out from 2003 to 2005 in the greenhouse complex of the "VU University Amsterdam". Two separate chambers, one with ambient CO_2 (±380 ppmv) and one with elevated CO_2 (±600 ppmv), were both connected to a temperature regulation system. Night and day temperatures were 10°C/15°C in winter and 15°C/20°C during summer, respectively. The temperature was gradually raised in spring or lowered in fall. The CO_2 was regulated year-round during daylight hours. The *B. pendula* saplings, half-siblings, were 2 years old at the start of the experiment. All individuals had one main stem without significant side branches and were between 50 and 80 cm long. They were planted in containers of 26 cm diameter and 22.5 cm height in April 2003. A double layer of root-cloth prevented roots from growing out of the drainage holes. They were planted in poor heath soil that originated from the province of Drenthe, the Netherlands. The soil pH was 4.5–5.0.

The full factorial setup of the experiment included two levels of CO_2 (ambient and 600 ppmv), three different forms of N additions (NH_4^+, NO_3^-, NH_4NO_3) and three different levels (0 kg N ha^{-1} $year^{-1}$, 50 kg N ha^{-1} $year^{-1}$, and 100 kg N ha^{-1} $year^{-1}$). Eight replicates (saplings in pots) were used per treatment. The 0 kg N ha^{-1} $year^{-1}$ treatments were used as a common control for the three different forms of N additions, resulting in a total of 112 trees. The three levels of N represent environments with very low N deposition (0) close to background levels, medium to high deposition (50), and very high deposition (100). These levels are based on present-day and historical deposition levels in the Nether-

lands. The N level of 100 kg ha^{-1} $year^{-1}$ is quite high, but has occurred in specific areas in the Netherlands during the 1980s and is not uncommon in agriculture. The 0, 50 and 100 kg N ha^{-1} $year^{-1}$ treatments will be referred to as control, 50-N, and 100-N, respectively. The N addition was applied in nine monthly portions each year, beginning just before the leaf buds opened (25th April 2003, 17th February 2004, and 18th February 2005). A 100-ml dilution of 1/10 Hoagland's nutrient solution without N (Hewitt and Smith 1975) was used as a base nutrient solution to which the experimental N was added in the form of NH_4Cl, $NaNO_3$, and NH_4NO_3. Subsequently, the plants were watered to field capacity with demineralised water and this was repeated in weeks without treatment. The position of the saplings in the greenhouse was changed every 2 weeks in 2003 and 2004, and every month in 2005 according to a randomization scheme. Pest control was performed both manually (once in 2003) and by spraying with Duoflor (once in 2003 and 2004).

Measurements

As this was a long-term experiment, any destructive growth or biomass measurements other than leaf sampling and length and stem thickness measurements were not performed during the first 2 years. In the last experimental year (2005) several plant parameters were determined. As a measure of biomass, the total dry weight of only the woody parts of the saplings (root and stem) was measured by harvesting. We assume that the responses of the total dry weight of woody parts to the treatments are representative for the responses of other biomass parameters (such as leaf biomass and total plant biomass, which were not measured). To determine the C and N concentration of green (sampled in June) and yellow leaves (sampled at various times during the end of the growing season), three air-dried leaves of each tree were randomly selected. The leaves were ground to a homogeneous powder with an Eppendorf grinder (Retsch MM301) using 2 ml Eppendorf containers and 5 mm diameter glass balls. Prior to analysis, the samples were dried at 70°C for 24 hours. Approximately 3 mg of the sample was weighed into tin foil containers (2×5 mm) and analysed for C and N concentration using a CHN elemental analyzer (Interscience CE instruments, EA 11110 CHNS-O). Senescence, the process that influences the yellow leaf stoichiometry is best understood by taking into account both nitrogen resorption efficiency (NRE) and resorption proficiency. Therefore, yellow leaf N concentration is depicted as N proficiency according to (Killingbeck 1996) and the mean NRE was calculated using the mean green and yellow leaf N concentration. According to Killingbeck (1996) N concentrations lower than 0.7% are indicative for complete resorption, between 0.7% and 1.0% indicate intermediate resorption, and >1.0% stand for incomplete resorption.

Statistical analysis

All results are or have been randomly cut down to an equal sample size which was $n=7$ for average combined total weight of root and stem, $n=5$ for average C:N of green leaves, and $n=4$ for average C:N of yellow leaves. The overall influence of CO_2, N type, and N level on all parameters was tested using analysis of variance (three-way ANOVA). In these analyses the N types were considered "unique". In case of interaction in the overall analyses, the data was split for N type and/or N level. To specifically test for differences between NH_4 and NO_3 treatments, separate variance analyses were performed on the data of these two treatments. The results of the statistical analyses are shown in Tables 1 and 2. Parameters were tested for normality and homogeneity of error variances. Deviations were, however, ignored because the data followed a symmetrical distribution and group sizes within the analysis were identical. Under these circumstances ANOVA is known to be robust against these violations. Differences were accepted as significant at $p<0.05$. All analyses have been performed using SPSS 11.5 for Windows.

Results

In the overall analysis, averaging all factors, elevated CO_2 increased the total dry weight of *B. pendula* saplings by 11% ($p<0.05$; Table 1). Increased N additions also resulted in a higher dry weight ($p<0.01$). Relative to the control, the 50-N and 100-N addition treatments increased the total dry weight by 122 and 147%, respectively. The type of N addition did not affect the dry weight. When tested for N levels

Table 1 Average values for total dry weight, green leaf N and yellow leaf C:N ratio and overall analysis

	CO_2	N-level	N-type
	380/600 ppmv	0/50/100 kg N ha^{-1} $year^{-1}$	Control/NH_4^+/NO_3^-/NH_4NO_3
Total dry weight (g)	102/113*	50/111/123**	50/120/113/119
Green leaf N (mg g^{-1} d wt)	23.7/18.6***	14.1/18.1/26.6***	14.1/21.3/23.4/22.4
Yellow leaf C:N ratio	69.4/79.1	91.5/85.6/57.0***	91.5/67.3/73.3/73.4

Average values for *B. pendula* total dry weight, green leaf N and yellow leaf C:N ratio and overall statistical analysis
*$p<0.05$; **$p<0.01$; ***$p<0.001$

separately (Table 2), it appeared that elevated CO_2 increased the total dry weight in the 50-N treatment, but not in the control-N or 100-N treatment. Also, in the 50-N treatment, there was a differential effect of the N-types on the total dry weight (trend towards increased total dry weight when NH_4^+ was given), which was absent at the 100-N level.

Elevated CO_2 resulted in an average overall reduction in green leaf N concentration of 21% compared to ambient CO_2 ($p<0.001$; Fig. 1, Table 1). N additions increased the green leaf N concentration ($p<0.001$), i.e., by 29% and 90% in the 50-N and 100-N treatments compared to the control, respectively. The split for N level (Table 2) showed that elevated CO_2 decreased the green leaf N concentration in the 50-N and 100-N treatment, but not in the control-N treatment. The overall analysis of the yellow leaf C:N ratio showed that N enrichment had a reducing effect ($p<0.001$; Fig. 2, Table 1). There was no difference between the control-N and 50-N levels, but the 100-N level was 38% lower than the control-N level and 33% lower than the 50-N level. The resorption proficiency is complete for both control treatments according to the levels given in Killingbeck (1996; Table 3). At the ambient CO_2 level, all 50-N treatments showed higher NRE values than the control, resulting in complete resorption proficiency. The ambient 100-N treatments however showed varying NRE values (higher, similar and lower than the control NRE) and all resulted in incomplete resorption proficiencies, shown by higher than normal N concentrations.

At elevated CO_2 two out of tree 50-N treatments had an NRE that was higher than the control NRE and all had complete resorption proficiencies. The NRE of two out of three elevated CO_2 100-N treatments were even higher than the control-N and 50-N levels, but in the end all had only intermediate or incomplete resorption proficiencies. There was no overall CO_2 effect.

The separate analysis with the data of the NH_4^+ and NO_3^- treatments showed that there was no difference in the effects of these two types on total weight or yellow leaf C:N ratio (data not shown). The effects on green leaf N concentration showed interaction with both CO_2 and N level. At ambient CO_2 the NO_3^- treatments yielded a higher green leaf N concentration at both N

Table 2 Average values for total dry weight, green leaf N and yellow leaf C:N ratio and detailed analyses

		CO_2	N-type
		380/600 ppmv	NH_4^+/NO_3^-/NH_4NO_3
Total dry weight (g)	Control	51.8/48.3	–
	50 kg N ha^{-1} $year^{-1}$	101/121***	119/107/107^
	100 kg N ha^{-1} $year^{-1}$	119/128	121/119/131
Green leaf N (mg g^{-1} d wt)	Control	14.9/13.2	–
	50 kg N ha^{-1} $year^{-1}$	20.6/15.6**	16.7/18.9/18.7
Yellow leaf C:N ratio	100 kg N ha^{-1} $year^{-1}$	29.7/23.5**	25.9/27.8/26.1
	Control	89.6/93.5	–
	50 kg N ha^{-1} $year^{-1}$	83.2/88.1	89.8/83.3/83.8
	100 kg N ha^{-1} $year^{-1}$	48.8/65.2	44.8/63.3/63.0

Average values for *B. pendula* total dry weight, green leaf N and yellow leaf C:N ratio and detailed statistical analyses
^$p<0.10$; *$p<0.05$; **$p<0.01$; ***$p<0.001$

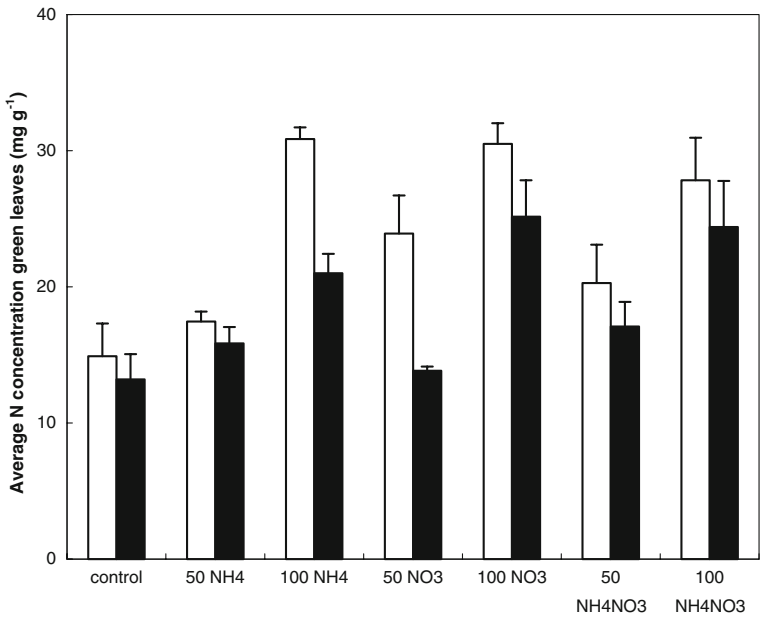

Fig. 1 Average N concentration of green leaves. Average N concentration of green leaves (mg g^{-1}±s.e.) of *B. pendula* saplings (n=5) in response to different N and CO_2 treatments. *White bars* represent ambient CO_2 (380 ppmv), *black bars* represent elevated CO_2 (600 ppmv). N doses (in kg N $ha^{-1}year^{-1}$) are indicated by 50 or 100, control stands for 0

levels. However, at elevated CO_2 the NO_3^- treatments yielded a higher green leaf N concentration only at the 100-N level. At the 50-N level, NH_4^+ resulted in a higher green leaf N concentration.

Discussion

The main questions raised in this study focused on the effects of elevated CO_2 and enhanced N availability on *B. pendula* NPP and leaf stoichiometry, and on the differential effects of inorganic nitrogen species on *B. pendula* functioning.

Effects on NPP

The first hypothesis, which stated that both elevated CO_2 and increased N availability separately would lead to a higher NPP and that the combination of both would lead to an even higher NPP, is only partly

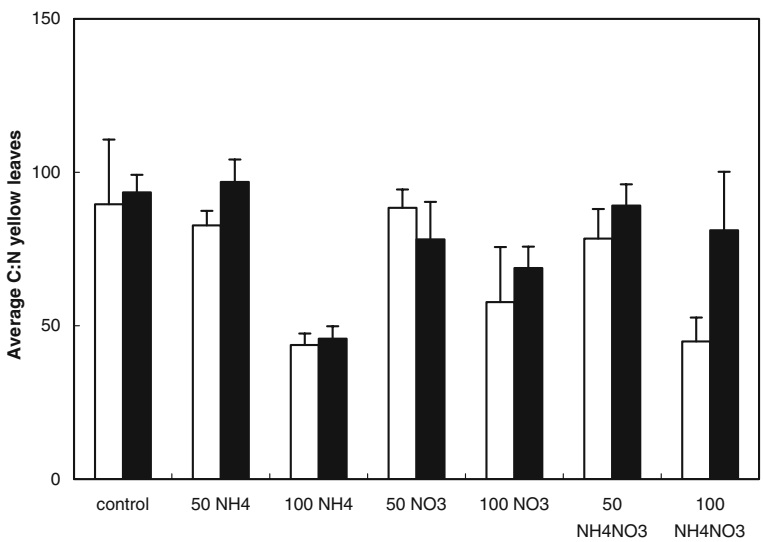

Fig. 2 Average C:N ratio of yellow leaves. Average C:N ratio of yellow leaves (±s.e.) of *B. pendula* saplings (n=4) in response to different N and CO_2 treatments. *White bars* represent ambient CO_2 (380 ppmv), *black bars* represent elevated CO_2 (600 ppmv). N doses (in kg N $ha^{-1}year^{-1}$) are indicated by 50 or 100, control stands for 0

Table 3 Average nitrogen resorption efficiency and resorption proficiency of yellow leaves

	380 ppmv CO_2				600 ppmv CO_2			
	NRE (%)	NRE relative to control	%N	Proficiency	NRE (%)	NRE relative to control	%N	Proficiency
Control	12.1		0.67	Complete	15.2		0.52	Complete
50 NH_4	21.0	+	0.62	Complete	20.1	+	0.53	Complete
100 NH_4	15.9	+	1.19	Incomplete	5.5	−	1.13	Incomplete
50 NO_3	31.5	+	0.59	Complete	10.2	−	0.69	Complete
100 NO_3	12.7	0	1.30	Incomplete	23.5	+	0.78	Intermediate
50 NH_4NO_3	19.6	+	0.69	Complete	19.4	+	0.58	Complete
100 NH_4NO_3	7.3	−	1.28	Incomplete	23.5	+	0.77	Intermediate

Average nitrogen resorption efficiency and resorption proficiency of *B. pendula* yellow leaves. N doses (in kg N ha^{-1} $year^{-1}$) are indicated by 50 or 100, control stands for 0. NRE stands for nitrogen resorption efficiency. NRE relative to control indicates whether the NRE is higher, neutral or lower than that of the control treatment. Proficiency is indicated as complete (%N<0.7), intermediate (0.7≤%N≤1.0) or incomplete (%N>1.0) according to Killingbeck 1996

supported by our data. The NPP is only raised by CO_2 when additional N is available. This result is not in agreement with two meta-studies where NPP (or growth/biomass) was increased by CO_2 irrespective of N level (Mcguire et al. 1995; Curtis and Wang 1998). However, in both these studies it was found that low nitrogen availability constrains the response of growth to elevated CO_2, which is corroborated by a study of Finzi et al. (2002). Here, in this experiment, the control treatment did not receive any N during the whole experiment apart from the initial soil N and some (indoor) background deposition. This N limitation probably prevented any biomass increase due to elevated CO_2. The absence of a CO_2 effect at the 100-N level is probably due to a limitation by one or more other elements, such as phosphorus or potassium. The increase in NPP by N addition in the N-amended treatments was, however, at least tenfold higher than the effect of elevated CO_2. A similar difference in effect size was also found by Lippert et al. (1996). When these results are interpreted in the context of the functioning of birch-dominated ecosystems, the effects of elevated CO_2 and increased N deposition on C and N cycling are highly dependent on the intensity of both the C and the N enrichment. Our results indicate that in ecosystems with very low atmospheric N deposition (such as the northernmost parts of Europe, North America and Asia) it is not very likely that elevated CO_2 alone will cause an increase in the biomass production. However, deposition is already increasing in these low-deposition areas. Ecosystems that already are subject to increased N deposition, have higher biomass production than before the onset of N enrichment. Elevated CO_2 will add to that increase. Elevated CO_2 (up to a certain level) leads to more biomass production, which implies that more C is fixed. Whether or not the additionally fixed C is retained within the ecosystem is dependent on the balance between biomass production and decomposition. The effects of elevated CO_2 on decomposition are related to the N richness of the system. C and N concentrations in green and yellow leaves can shed more light on this topic.

Effects on green leaf C:N

Increased N availability significantly increased the N concentration in the green leaves, as was also observed in several other studies (Ruohomaki et al. 1996; Tomassen et al. 2003). At the 50-N level this increase is accompanied by an increase in biomass while at the 100-N level only the green leaf N concentration increases. The increased N concentration is (partly for the 50-N treatment) the effect of luxury consumption of N, where N is stored as N-rich amino acids (Tomassen et al. 2003). Elevated CO_2 has, on the other hand, an opposite effect on N concentration in leaves, in some treatments even to the point where N-amended trees showed no difference compared with the control treatment (Fig. 1). Mcguire et al. (1995), as well as Poorter et al. (1997) reviewed the effects of elevated CO_2 and also found an overall decrease in green leaf N. This is most likely the result of N dilution by an increase in non-structural (Poorter et al. 1997) and structural carbohydrates at elevated CO_2. Green leaf N concentration

influences photosynthesis and palatability, but it does not influence the decomposition system directly (although N leaching from green leaves to the decomposition system will be positively related to N concentration). Leaves of deciduous trees withdraw nutrients from their leaves during senescence and the outcome of that process, the stoichiometry of yellow leaves, directly influences the decomposition process.

Effects on yellow leaf C:N

The hypothesis that yellow leaves grown at elevated CO_2 have a higher C:N ratio than those grown at ambient CO_2 is rejected. Though not significant, C:N was higher in almost all treatments, up to nearly 50% in the 100-NH_4NO_3 treatment. The absence of a significant CO_2 effect on yellow leaf C:N, while it was present in the green leaf C:N ratio, may indicate that the effect was indeed absent. However, because Coley et al. (2002) found an increase in yellow leaf C:N, and a meta-analysis by Norby et al. (2001) showed a decrease in yellow leaf N which is assumed to work through an increase in C:N, it could also be that the effect was there but that we have not been able to detect it. Firstly, because the yellow leaf C concentration was also reduced slightly (not shown), which may have (partly) leveled out the change in N concentration. Secondly, because the small number of replicates and relatively large variance combined resulted in relatively low statistical power in our analysis. Apart from this lack in CO_2 effect on yellow leaf C:N, there was also no difference in the onset and duration of senescence between the two CO_2 treatments.

The hypothesis that N enrichment would to lead to a lower yellow leaf C:N ratio, can be accepted. At the 100-N level either the withdrawal efficiency of N during leaf abscission was reduced, or the initial N concentration was so high that withdrawal was not sufficient to reach a minimum N concentration in the leaf. Therefore, we focus on the resorption proficiency and efficiency. Compared to other studies, see Aerts (1996) for a review, the NRE values found in this study are relatively low. This may be caused by our method; the autumn night temperatures in the greenhouse remained higher than outdoors and this may have influenced the NRE. However, although our values may need to be examined with care when compared to other studies, the relative differences between the treatments in our study remain valid. According to the resorption proficiency levels from Killingbeck (1996) resorption is complete for both control treatments. N is translocated to roots and stems at the end of the season to support growth in the next year, leaving only a minimum in the leaf. At the 50-N level, the NRE is higher than the relative control treatment in five out of six treatments. This higher NRE is, apparently, sufficient to reach N concentrations that are indicative for complete resorption. In four out of six 100-N treatments, N is translocated more efficiently or similarly to the control treatments, but this is not enough to reach complete proficiency as all 100-N treatments show intermediate to incomplete resorption proficiency. However, it seems that there may be a different response between ambient and elevated CO_2 conditions, with the latter yielding more intermediate N percentages.

Other authors have already indicated that nutrient translocation may proceed more efficiently under eutrophic conditions than under mesotrophic conditions(Chapin and Kedrowski 1983; Aerts 1990), probably due to the more mobile state of additionally formed N-rich amino-acids (Tomassen et al. 2003). This is (partly) corroborated by our data. The results seem to indicate that at ambient CO_2 very high N availability will cause a higher N concentration in yellow leaves. Elevated CO_2 combined with very high N availability will also cause a higher N concentration in yellow leaves, but to a lesser degree than at the ambient level. At the ecosystem level this indicates that at present-day CO_2, a moderately increased N deposition will not cause a decrease in the C:N ratio of the litter. However, at high N deposition levels, higher N concentrations are left in the leaf after senescence. These high N deposition levels were reached during the 1980s in the Netherlands, but may also occur in systems with dry deposition and a large aerodynamic roughness length such as forests and heaths (Erisman et al. 1998a). This can lead to a distinctly lower C:N ratio of litter that enters the decomposition system. Elevated CO_2 will not reverse this effect but may diminish it slightly.

Although decomposition is influenced by both biotic and abiotic factors such as pH, temperature, moisture and the presence of a microbial community, here we focus on the effects of litter quality characterized by the C:N ratio. While litter with a lower initial C:N ratio (or higher N concentration)

may or may not lead to a higher initial decomposition rate (Aerts and de Caluwe 1997; de Angelis et al. 2000; Norby et al. 2001; Limpens and Berendse 2003; Soudzilovskaia et al. 2007), Berg (2000) and Berg and Meentemeyer (2002) found that during the late stage of decomposition higher N concentrations retard the decomposition rate and that other elements such as calcium or manganese may influence the decomposition rate of foliar litter in later stages as well (Berg et al. 2007). Our study indicates that litter quality, measured as yellow leaf C:N, is enhanced at very high N availability irrespective of the effects of elevated CO_2. The values found, from above 90 for the control treatments to 57 for the 100-N treatments are expected to greatly increase the initial "digestibility" of the material for the decomposer community (Swift et al. 1979; Melillo et al. 1982), resulting in higher decomposition rates. However, in the long term, the initially higher N concentration may lead to a lower late stage decomposition rate (Berg 2000). This relation may possibly lead to, or add up to an increase in soil C storage in deciduous forest stands with very high N deposition (Berg and Meentemeyer 2002; Berg et al. 2007).

Effects of inorganic N species on biomass

The third hypothesis, that *B. pendula* will perform better when given NH_4^+ instead of NO_3^- has to be rejected. In the overall analysis, no parameter showed differential effects for the N types. This was confirmed by analysis of the data of solely the NH_4^+ and NO_3^- treatments. Apparently, *B. pendula*, which naturally grows on more or less acid and anaerobic soils does not prefer nor is more adapted to NH_4-N. Experiments on this subject show conflicting results. Generally, plants from circum-neutral, alkaline, and aerobic soils prefer NO_3^- as their N source, while those from acid and anaerobic soils often prefer NH_4^+ (Gigon and Rorison 1972; de Graaf et al. 1998; van den Berg et al. 2005a), although this is not always found (Crabtree and Bazzaz 1993). Other authors indicate that plant species of acidic soils where NH_4^+ is usually the predominant form of inorganic N (Troelstra et al. 1990) may be less affected by increased NH_4^+ concentrations, but that they do not perform better on either NH_4^+ or NO_3^- as their sole N source (Gigon and Rorison 1972; Falkengren-Grerup 1995). Our results indicate that elevated NH_4 deposition does not cause a larger NPP or biomass production for *B. pendula* than a similarly elevated NO_3^- deposition. At the ecosystem level this means that although wet and dry N deposition may show spatial variation in the composition in the dominant N type (NH_4^+ or NO_3^-), this will probably not cause differential effects on birch-dominated systems. Only in some specific cases, where birch species with a narrow pH range in the neutral to alkaline values occur (such as *B. alleghaniensis* with a pH range between 7 and 8), N deposition in the form of NH_4^+ may cause a species shift towards more acidic tolerant birch species.

Effects on the soil C storage

Soil C storage is the positive result of organic matter input and organic matter decomposition, and is influenced by multiple factors such as NPP, litter production and chemistry, mineralization, immobilisation, temperature, pH, moisture, nutrients and soil-organisms. Although the single-factor responses for most of these are known, they can be misleading when extrapolating to the real system because of in vitro- effects, short-term effects and (unknown) interactions and/or feedbacks (Hyvonen et al. 2007).

Although our results show that the combination of elevated CO_2 and increased N availability raised plant NPP and that the C:N ratio of leaf litter after senescence remained lower at high-N addition levels, we can only speculate on the effects on the soil C storage. Most straightforward, if nothing else changes, then increased litter production by the combination of elevated CO_2 and increased N could possibly result in an increased soil C storage (Schlesinger and Lichter 2001; Jastrow et al. 2005). The same is true if solely the effect of the lower C:N ratio on the decomposition rate is considered, because in that phase the formation of soil organic matter is negatively influenced by higher N concentrations (Berg 2000; Berg and Meentemeyer 2002). Also, if the higher availability of N (through higher N deposition) is followed by a decrease in soil pH, this may also decrease the decomposition rate or mass loss (Swift et al. 1979; Neuvonen and Suomela 1990) and increase soil C storage. In this chain of processes a higher atmospheric CO_2 concentration and elevated N deposition could therefore lead to an increase in the soil C storage.

However, this study only encompasses effects on Birch leaf litter, which makes up only a fraction of the total soil C input because soil organic matter input consists also from leachate, pollen, flowers, seeds, branches, stems and roots from several tree and other plant species. Also, less direct effects may lessen or reverse increased soil C storage such as the effect of a lower pH on the presence of decomposing fungi, bacteria or soil macro-organisms (Swift et al. 1979) or the effect of a higher atmospheric CO_2 concentration on temperature and therefore on the actual evapotranspiration (Aerts 1997) or (short or long-term) soil respiration (Melillo et al. 2002) for example. The final result on soil C storage is therefore unknown.

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